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Regular Article

Site-Specific Mutagenesis of Mistletoe Lectin: The Role of RIP Activity in Apoptosis*1

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Abstract

Recombinant mistletoe lectin (rML) belongs to the class of type II ribosome-inactivating proteins (RIP) composed of a catalytically active A-chain with rRNA N-glycosidase activity and a B-chain with carbohydrate binding properties. To investigate the contribution of the enzymatic activity of the rML A-chain to the observed cytotoxic and apoptotic effects, an rMLA E166Q R169Q molecule was developed by means of site-specific mutagenesis. Following heterologous expression, the activity of mutant rMLA was measured in a cell-free assay for rRNA–*N*-glycosidase activity. Moreover, after generation of heterodimer, the activities of mutant rML E166Q R169Q and rML wild type were determined in a cytotoxicity cytotoxicity and apoptosis assay. Although the reduction of activity as measured in the cell-free RIP assay was more pronounced (factor 237) than in both cellular assays (factors 20–22), the data clearly indicate a close correlation between cytotoxicity, apoptosis, and the enzymatic activity of the rML A-chain. Thus, RIP activity is an essential feature of rML and therefore a prerequisite for its biological function as an anticancer agent.

Author Keywords: *Viscum album;* ribosome-inactivating protein; viscumin; apoptosis; site-directed mutagenesis; *N*-riboside hydrolase (EC 3.2.2.22)

*1 Abbreviations used: rMLA, recombinant mistletoe lectin A-chain; rMLA E166Q R169Q, recombinant mistletoe lectin A-chain with modified active site; rML, recombinant mistletoe lectin heterodimer; rML E166Q R169Q, recombinant mistletoe lectin heterodimer with modified active site; pML, plant-derived mistletoe lectin I; MOLT-4, human T-cell leukemia line (ECACC No. 85011413); RIP, ribosome inactivating protein

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ELSEVIER SCIENCE FULL-TEXT ARTICLE

Crystal structure of mistletoe lectin I from Viscum album.

Krauspenhaar R, Eschenburg S, Perbandt M, Kornilov V, Konareva N, Mikailova I, Stoeva S, Wacker R, Maier T, Singh T, Mikhailov A, Voelter W, Betzel C.

Institute of Physiological Chemistry, University Hospital, c/o DESY, Build. 22a, Notkestrasse 85, Hamburg, 22603, Germany.

The crystal structure of the ribosome-inactivating protein (RIP) mistletoe lectin I (ML-I) from Viscum album has been solved by molecular replacement techniques. The structure has been refined to a crystallographic R-factor of 24.5% using X-ray diffraction data to 2.8 A resolution. The heterodimeric 63-kDa protein consists of a toxic A subunit which exhibits RNA-glycosidase activity and a galactose-specific lectin B subunit. The overall protein fold is similar to that of ricin from Ricinus communis; however, unlike ricin, ML-I is already medically applied as a component of a commercially available misteltoe extract with immunostimulating potency and for the treatment of human cancer. The three-dimensional structure reported here revealed structural details of this pharmaceutically important protein. The comparison to the structure of ricin gives more insights into the functional mechanism of this protein, provides structural details for further protein engineering studies, and may lead to the development of more effective therapeutic RIPs.

PMID: 10198229 [PubMed - indexed for MEDLINE]

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Eschenburg S, Krauspenhaar R, Mikhailov A, Stoeva S, Betzel C, Voelter W.

University Hospital c/o DESY, Building 22a, Notkestrasse 85, Hamburg, 22603, Germany.

The first three-dimensional structure of the ribosome inactivating protein mistletoe lectin I (ML-I) from Viscum album has been modeled on the basis of the X-ray structure of castor bean ricin from Ricinus communis. The relative high sequence homology and conserved secondary structure enabled accurate modeling. The 196 sequence changes between ML-I and ricin could be accomodated with only little pertubation in the main chain folding. A close comparison of the primary structures of ML-I and ricin is given and the effects of the sequence changes are elucidated on the basis of the modeled three-dimensional structure. Differences have been identified in the vicinity of the active site, in the high affinity galactose binding site and in the interface between the A and B chains, which might account for the reduced cytotoxicity of ML-I. Copyright 1998 Academic Press.

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